1 Introduction of Marine Algae Extracts

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1.1 Introduction

Recently, there is increased interest in naturally produced active compounds as alternatives to synthetic substances. Although these compounds often show lower activity, they are nontoxic and do not leave residues. It has already been reflected by the projects of new law regulations in EU countries that have imposed legal restrictions on the use of xenobiotics as plant protection products or preservatives. In the European Union there are plans of new directives that impose additional environmental taxes, primarily because of the residues of active substances in the environment. This implies that there is a need to develop new and safe products of biological origin, with properties similar to the synthetic, in particular antimicrobial, antifungal, antioxidizing compounds, and colorants. These natural compounds are found in algal extracts (Table 1.1).

Algal biomass have been used for centuries as food and medicine. The health promoting effects of algae were discovered as early as 1500 BC [1]. However, the biomass of algae gained interest as a source of chemicals and pharmaceuticals only recently. Nowadays, the production regime requires the use of extracts rather than the biomass itself, because of the formulation requirements (consistency, stability, color, flavor, etc.). Until now, algal products were available mainly as tablets, capsules, or liquid extracts, and sometimes were incorporated into food products, cosmetics, or products for plants [2]. In 2006, the market of microalgal biomass produced 5000 mg dry biomass/year and generated a turnover of $1.25 \times 10^9$ USD [2]. The global sector of macroalgae is worth 6 billion USD, with main contribution from hydrocolloids and crop protection products [3]. Recently, compounds derived from algae (carotenoids, β-carotene astaxanthin, long-chain polyunsaturated fatty acids (PUFAs), docosahexaenoic acid) began to be produced on industrial scale [4]. Novel compounds isolated from algae possess a great further potential to be applied for their pharmacological and biological activity [4].

Seaweeds produce a vast spectrum of secondary metabolites because they live in nonfriendly environment but are not damaged photodynamically as they synthesize protective compounds and develop protecting mechanisms.
### Table 1.1 Major compounds in algal extracts [2, 11, 19, 20].

<table>
<thead>
<tr>
<th>Compound</th>
<th>Function</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polysaccharides</td>
<td>Components of cell wall (fucoidan, alginate, laminarin)</td>
<td>Provide strength, flexibility, maintain ionic equilibrium, prevent from desiccation</td>
</tr>
<tr>
<td>Phenolics and phlorotannins</td>
<td>Not found in terrestrial plants Phenol rings in polyphenols act as electron traps to scavenge radicals</td>
<td>Antimicrobial, antioxidant, antiviral compounds that protect the algae from abiotic and biotic stress conditions, for example, phlorotannins that are formed from oligomeric structures and phloroglucinol</td>
</tr>
<tr>
<td>Protein, peptides, and essential amino acids</td>
<td>The contents vary</td>
<td>Antioxidative, but difficult to extract</td>
</tr>
<tr>
<td>Lipids</td>
<td>Polyunsaturated fatty acids (PUFA) (ω-3 and ω-6) – higher level than in terrestrial plants</td>
<td>Structural membrane lipids; important in human and livestock diet. Composed of glycerol, sugars, bases esterified with fatty acids (saturated or unsaturated (C12–C22))</td>
</tr>
<tr>
<td>Terpenoids and steroids</td>
<td>Carotenoids, xanthophyll, fucoxanthin, astaxanthin</td>
<td>Antioxidant, antiviral, anti-inflammatory activity, UV protection</td>
</tr>
<tr>
<td>Vitamins</td>
<td>A, B1, B2, B6, B12, C, E, nicotinate, biotin, folic acid, pantothenic acid. Level depends on the season</td>
<td>—</td>
</tr>
<tr>
<td>Minerals</td>
<td>Se, Zn, Mn, Cu – structural components of antioxidative enzymes</td>
<td>—</td>
</tr>
</tbody>
</table>

[5]. Environmental stress to which algae are exposed include rapid fluctuations of light intensity, temperature, osmotic stress, desiccation that lead to the formation of free radicals and oxidizing agents that lead to photodynamic damage [6].

### 1.2 Algal Biomass as a Useful Resource

Algae are the oldest photosynthetic organisms dating back to 3.8 billion years (prokaryotic cyanophytes) [7]. The number of species is estimated as 280,000 [7]. Algal biomass is being used as the raw material for different branches of industry.
and the global production is prevalently increasing [7]. Algae are photosynthetic organisms that convert light energy from the Sun into chemical energy stored in the form of chemical compounds in the process of photosynthesis [1]. A characteristic of algae is that they possess a simple reproductive structure [8]. The biomass of algae contains various compounds with diversified structures and functions that are synthesized in the response to stress conditions, for example, heat/cold, desiccation, salinity, osmotic stress, anaerobiosis, nitrogen deficiency, photooxidation, as protection from physiological stressors [1]. Algae are a diversified group of organisms and are divided into microalgae and macroalgae. The first group includes prokaryotic cyanobacteria and eukaryotic microalgae [9]. Algae are very diversified organisms when considering size (from unicellular microalgae to multicellular macroalgae) [10].

The classification of algae is pigmentation: green (Chlorophyceae), red (Rhodophyceae), and brown (Phaeophyceae) [11]. The difference concerns not only pigmentation, but also the type of storage material and the composition of cell wall polysaccharides [12]. Algae are simpler than terrestrial plants [12]. Algae could be considered as natural factories that produce bioactive compounds [13]. The composition of green algae: 10% protein, 35% carbohydrate, and 50% ash (Ca, Fe, P, Cl) [12].

Algae were in use since prehistory as the components of diet and as medicine [14]. Although the importance of algal industry is permanently increasing, there are some contradictions between Asian (Far East) and European ways of utilization of this resource [14]. In Europe, the biomass of seaweed was treated as a sort of waste from seas and oceans [14]. Certainly, algal biomass is still an underutilized biological resource.

Algal biotechnology is divided into two branches: microalgal and macroalgal, with its unique specificity [15]. Microscopic algae are called microalgae; however, this term is not related to taxonomy. Among microalgae, cyanobacteria are distinguished, which are prokaryotic [15]. Macroalgal biotechnology includes the production of phycocolloids agar-agar, alginates, carrageenan from Rhodophyta and Phaeophyta, and the global value is $6 \times 10^9$ per year [15]. At present, the main directions in macroalgal biotechnology are biofuels, agricultural biostimulants for crop plants, probiotics for aquaculture, soil bioremediation, wastewater treatment, and biomedical applications of extracted compounds (polyphenols, polysaccharides) [3]. Microalgal biotechnology refers to the production of different products: phycocyanin, carotenoids (β-carotene, astaxanthin), fatty acids and lipids, polysaccharides, immune modulators that find an application in health food, cosmetics, feed and food supplements, pharmaceuticals, and fuel production [15]. Microalgal groups of the major importance are cyanobacteria (Spirulina sp.), Chlorophyta (Chlorella sp., Dunaliella sp.), Rhodophyta (Porphyridium sp.), Bacillariophyta (Odontella sp., Phaeodactylum sp.) [15]. While macroalgae are harvested from natural habitats, microalgae are cultivated in artificial systems [15]. The products of microalgal biotechnology are coloring substances (astaxanthin, phycocyanin, phycoerythrin), antioxidants (β-carotene, tocopherol, antioxidant CO2 extract), and arachidonic acid (ARA), docosahexaenoic acid (DHA), and PUFA extracts [15].
1.3 Biologically Active Compounds Extracted from Algae

Because algae are coastal primary producers and have impressive possibilities to survive in extreme environmental conditions, in particular to trigger oxidative stress, they produce a variety of useful compounds [16]. Algae live in extreme conditions: fluctuating salinity, temperature, nutrients, and UV–vis irradiation [10]. Long periods of desiccation cause overproduction of reactive oxygen species, which is neutralized by physiological and biological mechanisms: the production of secondary metabolites [16]. Therefore, compounds isolated from the biomass of seaweeds possess biological activity. The biomass of algae contains many valuable components: minerals, vitamins (A, B, C, E), PUFAs (ω-3), amino acids, proteins, polysaccharides, lipids, and dietary fiber [17]. Many of these bioactive constituents can be extracted to obtain antioxidative, anti-inflammatory, antimicrobial, anticancer, antihypertensive products [11, 17]. Particularly useful are secondary metabolites with antiviral, antimalarial, anticancer properties [1]. Products derived from algae also contain polysaccharides, polyphenolic compounds, and terpenes [11]. Seaweeds and their extracts are added to food as antioxidants, antimicrobials, dietary fiber, and dietary iodine [6].

In various studies, strong antioxidative properties of compounds isolated from seaweeds were confirmed [18]. Antioxidative activity produces phlorotannins (polyphenolic compounds – 1–10% d.m. of brown seaweeds), alkaloids, terpenes, ascorbic acid, tocopherols, and carotenoids [18]. Antioxidants transform radicals into nonradicals by donating electrons and hydrogen, chelation of transition metals, and dissolving peroxidation compounds [6]. The role of antioxidants is to prevent lipid oxidation, inhibiting the formation of products as a result of oxidation, and consequently prolonging the shelf life of products [6]. Algae are a rich source of natural antioxidants and antimicrobial compounds [6].

The research on the composition of algal extracts concerns mainly antioxidants as an alternative to synthetic, because according to recent research these compounds if used as food additives are potential promoters of carcinogenesis [1]. The extracts modulate the oxidative stress and diseases related to radical scavenging effect: sesquiterpenoids and flavonoids (green alga Ulva lactuca), phlorotannins (brown alga Eisenia bicyclis, Ecklonia cava, E. kurome), phycobiliprotein, and phycocyanin (blue-green alga Spirulina platensis), which protect from DNA damage by H$_2$O$_2$ [17].

- Anti-HIV – cyanovirin – protein from Nostoc ellipsosporum [1]
- Photoprotective compounds – repair DNA damage – mycosporine-like amino acids, scytonemin enzymes (shock proteins) – superoxide dismutase, catalase, and peroxidase [1].

Microalgae contain carotenoids, PUFAs, phycobilins, sterols, polyhydroxyalkonates, and polysaccharides [9]. They can be considered as cosmeceuticals, nutraceuticals, and functional foods [9]. For instance, Spirulina contains lipids
1.4 The Application of Products Derived from Algal Biomass

(6–13; 50% in the form of fatty acids), phycocyanin (20–28%), and carbohydrates (15–20%; mainly as polysaccharides) [21].

Algal cells contain phytochelatins – proteins synthesized in response to exposure to toxic metal ions [22]. However, the attempt to extract and use these proteins is not found in the available literature [22].

1.4 The Application of Products Derived from Algal Biomass

The global wild stocks of seaweeds yield 8 million mg of biomass [18]. In 2004, the contribution in the market was as follows: sea vegetables (88%), phycocolloids (11%), phycosupplements (1%), and the minor contribution of soil additives, agrochemicals, and animal feeds (totally, 6000 million USD) [14]. Algal extracts create a new market sector, because they can be used in a variety of products, for example, antioxidant capsules containing Spirulina extract, Chlorella extract in health drinks, oral capsules containing carotenoid extracts from Dunaliella [15]. Other examples of algal extracts-based products are pet functional food, biofertilizers (which increase water-binding capacity and serve as the source of minerals and substances promoting germination, growth of leaves and stems and flowering).

Of particular interest are antioxidants present in algae and their extracts, as the use of synthetic antioxidants has been restricted because of toxicity and health risks [23]. It is important to replace these synthetic compounds with natural antioxidants [23]. Antioxidative compounds from marine sources include various functional compounds, for example, tocopherols [19]. Lipid-soluble algal extracts can be used as protective functional ingredients [19]. Antioxidative properties of natural compounds from algae can prolong the shelf life of foods and cosmetics through delayed oxidation [11]. Natural anti-oxidants may also be useful in treating aging, UV-exposure, and diseases associated with oxidation [11]. Extracts from algae are used in cosmetics, for example, from Spirulina and Chlorella [2].

Polysaccharides isolated from algae are other important components of foods and cosmetics and in nutraceutical and pharmaceutical preparations and are produced mainly from seaweeds [21]. Polysaccharides (carrageenans, alginates) are used in food industry as edible packaging materials [6].

The main source of industrially exploited polysaccharides (alginate, agar, carrageenan) originates from the biomass of algae [12]. Algal biomass contains significantly higher levels of polysaccharides than terrestrial plants [12]. Algal polysaccharides differ from those in terrestrial plants: sulfate groups, additional sugar residues, high content of ionic groups, high solubility in water, and unique rheological properties [12].

Polysaccharide production includes the following steps: selection of raw material, stabilization and grinding of biomass, extraction and purification, precipitation, and drying [12].
1.4.1
Agriculture – For Plants

In modern agriculture, higher production should accompany lower environmental impact and higher sustainability [24]. These criteria fulfill biostimulants that improve efficiency of regular fertilization (increase the efficiency of nutrients uptake), enhance yield and the quality of crops, improve tolerance to environmental stress, and possess antioxidant properties [24]. Biostimulants are natural substances that promote growth, uptake of nutrients, and tolerance to abiotic stress and different climatic conditions [25]. Seaweed extracts can be used as foliar sprays for vegetables, grains, and flowers [24]. Plant growth regulators are defined as bioactive compounds. It is desired that they perform well and are degraded into products that are not harmful to the environment [26].

The European Biostimulant Industry Council (EBIC) was established to help introduce agriculture biostimulants to the market and support regulatory EU authorities to describe biostimulants as innovative class of products, the production of which uses minimal synthetic processing. Biostimulants are approved in organic crops, with an important group of products derived from macroalgae [27].

Seaweeds have been used in the cultivation of plants since antiquity [28]. Seaweeds were composted since antiquity and used as soil amendments. The first industrial applications of seaweeds in agriculture were in 1944, as the new source of fiber [14]. At present, the extracts are applied directly to shoots foliarly or to soil [3]. The examples of algal extracts currently available on the market are Kelpak, Actiwave, and AlgaGreen [3]. Seaweed concentrates (e.g., Kelpak) are applied at low rates and have growth promoting effect following the presence of plant growth regulators (e.g., cytokinins and auxins, polyamines, putrescine, spermine) rather than nutrients [29]. These active substances increase the growth of nutrient-stressed plants [29].

In 1949, the product Maxicrop was developed [14]. Using liquid seaweed is advantageous, because plants respond immediately and positively (dilution 1:500); also, the ions of micronutrients (Cu, Co, Mn, Fe) are soluble at high pH and are chelated by partly hydrolyzed sulfated polysaccharides; soil crumb structure is improved (with alginate and fucoidan), microorganisms, root system, and plant growth are stimulated [14].

Extracts from seaweeds are useful in the cultivation of plants because they improve a wide range of physiological responses: increase crop yield, improve growth, improve plants’ resistance to frost, serve as biofungicide and bioinsecticide, increase nutrients’ uptake from soil because they contain plant growth regulators [30]. The extracts are used in low doses (high dilutions), because the active substances are efficient even in small quantities [30].

The compounds found in algal extracts that are important for plant growth are cytokinins, auxins, abscisic acid, vitamins, amino acids, and nutrients [24]. The outcome is the result of the synergistic effect of many compounds present in algal extracts [24]: phytohormones, betaines (organic osmolytes), polymers, nutrients, and alginic acid (soil conditioning agent that supports soil structure) [25, 28].
There are various reports of laboratory, pot, and field studies that aimed to test the plant growth stimulating properties of algal extracts. El-Baky et al. [31] investigated the effect of treatments with microalgae extracts (Spirulina maxima and Chlorella ellipsoida) on antioxidative properties in grains of wheat. The content of carotenoid, tocopherol, phenolic, and protein in grain was investigated. Antioxidant activity of ethanolic extracts showed the significant increase of radical scavenging activity in response to microalgal extracts treatment [31].

**1.4.2 Functional Food**

Functional food is defined as food that positively affects one or more physiological functions to increase the well-being and reduce the risk of suffering for diseases [8]. Recently, a new market for functional food has evolved, the food called “food for the not-so-healthy” [13]. Functional food is produced by the addition of active components. Functional food contains functional ingredients: micronutrients ω-3 fatty acids, linoleic acids, phytosterols, soluble fiber (inulin – prebiotics), probiotics, carotenoids, polyphenols, vitamins that present healthy effect on the organism [13]. New, biologically active natural ingredients (antioxidant, antiviral, antihypertensive) extracted from the biomass of algae are becoming important research objects in the area of food science and technology [10].

Algal extracts are the components of functional food, because they are considered as natural, biologically active components. The latter, beside nutrition, should have the beneficial influence on functions of the body by improving health or preventing from diseases [32]. Extracts from Spirulina can be added to functional foods because of antioxidant, antimicrobial, anti-inflammatory, antiviral, and antitumoral properties of the compounds (phytocyanins, carotenoids, phenolic acids, and ω-3 and six PUFAs) [32].

Algae are used as dietary supplements that are classified into three groups: (i) Spirulina platensis, (ii) Aph. flos-aquae, and (iii) Chlorella pyrenoidosa [33]. The biomass of these microalgae is obtained either from lakes or by cultivation in artificial ponds [33]. Algae can be cultivated, in which the growth rate is high and in some cases there is a possibility of controlling the production of active compounds by adjusting cultivation conditions [10].

The potential use of brown seaweed extracts to inhibit the growth of microorganisms responsible for food spoilage and pathogenic microorganisms was also investigated [5]. The addition of 6% of the extract substantially reduced the growth of nondesired microflora [5].

**1.4.3 Cosmetics**

Microalgae, the biomass of which is to be used as the raw material for isolation of beneficial compounds, are cultivated in artificial systems that provide the biomass that is free of impurities [7]. Algal extracts are useful in the skin care market as
well because they support regeneration of tissues and reduce wrinkles, in particular, the extracts from *Spirulina* (which repair signs of aging, prevent stria formation) and *Chlorella* (stimulate collagen synthesis) [2]. The properties of microalgal extracts include reduction of intracellular oxidative stress and synthesis of collagen [7].

Extracts from the following microalgae are produced commercially for cosmetic industry [7]:

- *Nannochloropsis oculata* – vitamin B12, vitamin C, and antioxidants
- *Dunaliella salina* – pigment industry (carotenes), amino acids, and polyphenols
- *Chlorella vulgaris* – proteins, and inorganics substances.

### 1.4.4 Pharmaceuticals

Algal extracts can replace commercial antibiotics in disease treatments [34]. Biologically active metabolites isolated from marine algae have the potential to be used as pharmaceuticals because they inhibit the growth of bacteria, viruses, and fungi [34]. The chemicals are macrolides, cyclic peptides, proteins, polyketides, sesquiterpenes, terpenes, and fatty acids [34]. Cavallo et al. [34] investigated the effect of lipid extracts from six algae and their antibacterial activity against fish pathogens and found that they can be used as antibacterial, health promoting feed for aquaculture.

Extracts from *Spirulina* are active against viruses (herpes, influenza, cytomegalovirus) and inhibit carcinogenesis [35]. *Spirulina* is the source of vitamin A that is highly absorbable [36].

Hot water extract from *Spirulina* supports human immune system by the improvement of immune markers in blood (higher level of gamma interferon and interleukin-12p40 and toll-like receptors) and acts directly on myeloid lineages and natural killer-cells (NK cells) [35]. Immulina is a polysaccharide found in the extract from *Spirulina* that activates monocytes. Water extracts also showed antiviral activity [35].

### 1.4.5 Fuels

Seaweed extracts can be the resource to produce liquid fuels (ethanol), because of high carbohydrates (laminaran, mannitol) content [37]. Seaweeds can be bioconverted to methane [37].

### 1.4.6 Antifouling Compounds

Extracts from marine algae (e.g., *Enteromorpha prolifera*) contain compounds that have antifouling properties toward, for example, mussels (*Mytilus edulis*) and
larval settlement: tannins (*Sargassum natans*), bromophenol (*Rhodomela larix*), diterpenes (*Dictyota menstrualis*), and halogenated furanones (*Delisea pulchra*). These compounds have the potential in the prevention from fouling of ship hulls and aquaculture nets instead of organotin or paints based on toxic metals [38].

### 1.5 Extraction Technology

Seaweed industry was established in 1950s [3]. The production concerned mainly low-cost fertilizers and food [3]. For the first time liquefaction of seaweeds was undertaken in 1857 by compressing [28]. The goal was to obtain the formulation that is transportable over long distances [28]. Algal extracts were obtained and patented in 1952 by alkaline extraction [3]. Another process was milling in low temperature [28].

Although natural extracts possess a great applicable potential, the problem with natural products is variable composition of extracts because of fluctuations in the raw material (season, location), different extraction techniques [12]. Extraction methods vary and the following can be distinguished: ethanol, methanol, enzymatic [17], composting, supercritical CO$_2$ extraction with cosolvents.

In the elaboration of a new extraction technology, it is necessary to select the target bioactive compound, select the species of alga for extraction containing the compound of interest, select the operation conditions to find a compromise between the yield and purity, and consider if large enough resources of the algae are available.

It is essential to develop appropriate, quick, cost-efficient, and environmentally friendly methods of extraction that aim to isolate biologically active compounds of interest [10] without loss of their activity. It is essential to develop extraction procedures that involve the use of specific solvents and processes [8].

The production of algal extracts consists of several unit operations [7]:

- Upstream processing – preparation for cultivation
- Cultivation – in photobioreactors
- Downstream processing – cell harvesting, rehydration and hot water extraction, centrifugation, and ultrafiltration
- Formulation, preservation, and conditioning.

Traditional extraction techniques (soxhlet) solid–liquid extraction (SLE), liquid–liquid extraction (LLE) consume large quantities of solvents and require high extraction times [8]. These procedures present low yield of extraction and low selectivity toward bioactive compounds [8]. Because of the lack of automation, reproducibility is low [8]. Recently developed techniques supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), accelerated solvent extraction (ASE), pressurized hot water extraction (PHWE), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE) have further reduced these limitations [8]:


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- **Solvent extraction** – large quantities of toxic organic solvents are used, long time of extraction, laborious, low selectivity, low extraction yields, and not mild conditions (temperature, light, oxygen) [32].
- **Pressure liquid extraction** – less solvent, shorter time of extraction, automated, no oxygen, and no light [32].
- **Supercritical fluid extraction** – technique used to isolate active components from natural materials [32].

SFE uses solvents at temperatures and pressures above their critical point and is used to extract compounds from biomasses [8]. In this technique, the consumption of toxic organic solvents is reduced and the main solvent used is CO\textsubscript{2} [8]. The disadvantage is low polarity of CO\textsubscript{2} and resulting necessity of the use of polar modifiers or cosolvents [8]. Advantages are high diffusivity, easiness in the control of temperature and pressure (possibility of modification of solvent strength), and obtaining solvent-free extracts [8].

Extraction of biologically active compounds from algal biomass is not selective. The extract is a mixture of different compounds [11]. The factors that influence the composition and thus the activity of algal extracts depend on species, environmental conditions, season of the year, age, geographical location, and processing technologies [11]. For instance, ethanol was found to be more efficient in the extraction of polyphenols than water [23]. Seaweed extracts contain PUFAs (in particular ω-3 long chain PUFA) that have several health promoting effects and have the potential to be useful in treatment or reducing symptoms of: cardiovascular disease, depression, rheumatoid arthritis, and cancer [19].

Chaiklahan et al. [21] optimized the extraction of polysaccharides from *Spirulina* sp. It was found that the mostly significant operation conditions were temperature and solid to liquid ratio and time. The extract contains rhamnose and phenolic content [21].

Seaweed concentrates are used as supplementary soil conditioners that promote plant growth and improve crop yield [29]. An example product is Kelpak\textsuperscript{®} from *Ecklonia maxima* [29]. These products are used in very low doses and contain, for example, cytokinins and auxins that are plant growth regulators [29]. Seaweed extracts are particularly useful if applied on plants that are nutrient-stressed [29].

1.6 Conclusions

Algae are a useful raw material for biobased economy, because their cells contain a vast array of useful compounds with high biological activity. Biomass of algae is certainly an underestimated resource. In the process of extraction it is possible to draw the valuable compounds closed in the algal cells. However, this should be carried out in such a way that the structure and thus the properties of the compounds are not destroyed and that the solvent used does not limit their use as safe components of products for plants, animals, and human.
There are many ways to implement the extraction process and this is thoroughly discussed in this book. In addition to developing extraction technology, it is very important to assess the utilitarian values of the extracts, which can be documented in application studies of extracts in real systems.

Preparation of algal extracts represents a new approach in the preparation of natural products with a standardized composition, as compared with the biomass itself and certainly will be a future for algal industry.

References


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